

Supporting Information

Strains and Expression Plasmids. The strain M15MA was constructed using P1 phage-mediated generalized transduction. Briefly, P1 phage were used to infect strain CAG 18491 (Yale *E. coli* Genetic Stock Center) which contains the *metE*::Tn10 mutation. The phage collected from infected CAG 18491 cells were used to transduce strain M15 (Qiagen Corp., Valencia, CA). A stabilization procedure¹ was followed to eliminate the transposon and create a stable auxotroph. The gene encoding OmpC was amplified from M15MA genomic DNA using the following primers: OmpC for, 5'-CTGCGCCTGGTCTCACATGAAAGTTAAGTACTG-3' and OmpC rev, 5'-CCGAAGCTTTTATTAGAACTGGTAAACCGG-3'. The PCR product was sequentially digested with *Hind*III and *Bsa*I and subsequently ligated to pQE-60 (Qiagen) which was digested with *Nco*I and *Hind*III. The resulting plasmid, pQE-60 OmpC, was transformed into competent M15MA cells harboring the plasmid pREP4, generating the expression strain M15MA[pQE-60/OmpC].

Site-Directed Mutagenesis. Mutations introduced to OmpC to enrich its methionine content were as follows: V50M, N88M, T187M, A230M, L271M, and L306M. The mutations were introduced using a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's recommendations.

Synthesis of Azidohomoalanine and Biotin-PEG-Propargylamide.

Azidohomoalanine (**1**) was prepared as previously described.² Biotin-PEO-propargylamide **2** was prepared by dissolving TFP-PEO-Biotin (32 mg, Pierce, Rockford, IL) in excess neat propargylamine. After 10 minutes, the solution was added dropwise to ethyl ether. A white precipitate formed and was collected by centrifugation. The precipitate was dried *in vacuo* yielding 22 mg (82 %) of **2**. This material was characterized by ¹H and COSY NMR to confirm the formation of an amide bond. ¹H NMR (CD₃OD): δ 1.38-1.50 (m, 2H), δ 1.51-1.88 (m, 8H), δ 2.19 (t, 4H, *J* = 7.3 Hz -CO-CH₂-CH₂-CO-), δ 2.46 (t, 2H, *J* = 2.7 Hz, -CH₂-CO-NH-) δ 2.58 (t, 1H, *J* = 2.7 Hz, -NH-CH₂-C≡C-H), δ 2.89-2.96 (dd, 2H, *J*₁ = 5.1 Hz, *J*₂ = 12.5 Hz, CH-CH₂-S), δ 3.17-3.28 (m, 5H), δ 3.44-3.69 (m, 12 H), δ 3.93 (d, 2H, *J* = 2.7 Hz, -NH-CH₂-C≡C-H), δ 4.27-4.35 (dd, 1H, *J*₁ = 5.1 Hz, *J*₂ = 7.9 Hz, -HN-CH-CH₂-), δ 4.46-4.54 (dd, 1H, *J*₁ = 4.5 Hz, *J*₂ = 7.9 Hz, -HN-CH-CH-).

Metabolic Incorporation of Azidohomoalanine into OmpC. A single colony of M15MA[pQE-60/OmpC] was grown overnight in M9 minimal medium supplemented with all twenty natural amino acids and the antibiotics ampicillin and kanamycin. A small amount of this starter culture was added to fresh M9 medium supplemented with all of the natural amino acids. Upon reaching an OD₆₀₀ of 1, the cells were pelleted and resuspended in M9 medium containing all natural amino acids except for methionine. After agitation at 37 °C for 10 minutes, the cells were pelleted again and resuspended in M9 medium with 19 amino acids (no methionine). The cells were divided into three equal portions:

one for methionine, one for azidohomoalanine, and one for no analog. Methionine and azidohomoalanine were added to the appropriate cultures at a concentration of 40 mg/L. Isopropyl- β -D-thiogalactoside (IPTG) was added to a final concentration of 1 mM to induce expression of OmpC. Induction at 37 °C was for three hours.

[3+2] Cycloaddition Chemistry on Whole Cells. Cells producing recombinant OmpC (1 mL culture) were pelleted and washed once with PBS (pH 7.4) and resuspended in 1 mL PBS. The [3+2] cycloaddition conditions were essentially as described previously.³ Specifically, CuSO₄ was added to a final concentration of 100 μ M while TCEP and the triazole ligand were added to a final concentration of 200 μ M. The final concentration of biotin-PEO-propargylamide was 50 μ M. Given that each cell expresses $\sim 10^5$ copies of OmpC,⁴ and the reaction was performed on $\sim 10^9$ cells in a volume of 1 mL, this concentration of biotin-PEO-propargylamide represents approximately a 100-fold excess of alkyne to azide. The reaction was allowed to proceed for 16 h at 4 °C with agitation. At the conclusion of the reaction, the cells were pelleted and washed twice with PBS to remove excess reagents.

Purification of Outer Membrane Proteins and Western Blotting. The outer membrane protein fraction of cells functionalized with biotin-PEO-propargylamide was prepared as previously described.⁴ Purified outer membrane fractions were electrophoresed (12 % tris-tricine gel, 150 V) and transferred to a nitrocellulose membrane (30 V, 1 h at 4 °C). The membrane was blocked with a 5 % milk solution in PBS/Tween for 1 h. Following washing, the membrane was probed with an avidin-HRP conjugate (Amersham Biosciences, Piscataway, NJ) and the bands were visualized with detection reagents (Amersham Biosciences).

Flow Cytometry. Analysis of cells by flow cytometry was carried out on a DakoCytomation MoFlo cell sorter (Fort Collins, CO) equipped with a 488 nm laser. Cells functionalized with biotin-PEO-propargylamide were incubated with an avidin-Alexa Fluor 488 conjugate (Molecular Probes, Eugene, OR) for two hours at 4 °C. After avidin staining, the cells were washed three times with PBS and diluted to a density of approximately 2×10^7 cells/mL. Between 20,000 and 50,000 events were gathered in each experiment. The data was analyzed using Summit Software (DakoCytomation, Ft. Collins, CO).

¹ Bochner, B. R., Huang, H., Schieven, G. L., Ames, B. N. *J. Bacteriol.* **1980**, *143*, 926.

² Mangold, J. B., Mischke, M. R., LaVelle, J. M. *Mutat. Res.* **1989**, *216*, 27.

³ Wang, Q. Chan, T., Hilgraf, R., Fokin, V. V., Sharpless, K. B., Finn, M. G. *J. Am. Chem. Soc.*, **2003**, *125*, 3192

⁴ Xu, Z. and Lee, S.Y., *Appl. Environ. Microbiol.* **1999**, *65*, 5142.